EFFECT OF EXTRUSION FEED PROCESSING ON EQUINE DIGESTION

A Thesis

by

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ABSTRACT

Nineteen Quarter horse geldings (466 to 697 kg BW; 9 to 18 yr age) were used in a randomized design to determine the difference in nutrient utilization of either pelleted or extruded concentrate. Horses were randomly assigned to one of two treatments: a pelleted diet (PEL; n = 10) or an extruded diet (EXT; n = 9, Life Plus, Muenster Milling, LLC, Muenster, TX). Diets were formulated to be isocaloric and isonitrogenous with 8% fat and 14% protein, respectively. Dietary treatments were fed at 0.5% BW (as fed) daily, and all horses received 1.5% BW coastal bermudagrass (*Cynodon dactylon*) hay (as fed) daily. Horses were fed at 0630 and 1830 h daily, and refusals (ORTs) were collected and weighed 3 h after feeding.

The first 14-d were used as a dietary adaptation period, followed by 4-d total fecal collection. Fecal samples were collected from fecal harnesses (Bun-bag, Sagle, ID) every 6 h, weighed, homogenized, and subsampled. Additionally, concentrate rate of intake was measured over 5 feedings and averaged to determine kg consumed/min.

Data were analyzed using PROC MIXED procedure of SAS. Concentrate rate of intake was influenced by treatment ($P = 0.05$) with PEL eating at 0.16 kg/min and EXT eating at 0.13 kg/min. Concentrate dry matter intake was greater ($P = 0.02$) for EXT than PEL (0.46 and 0.44%, respectively) this difference resulted from a higher DM content for the EXT than the PEL. There were also no differences in hay intake ($P = 0.70$), total dry matter intake ($P = 0.99$), digestible dry mater intake ($P = 0.17$), or starch intake ($P = 0.62$). Dry matter digestion was greater ($P = 0.03$) for PEL at 51% compared to 48% for

EXT. In accordance, organic matter digestion followed a similar response ($P = 0.03$) with digestibilities of 51% and 48% for PEL and EXT, respectively. Starch digestion was greater ($P = 0.02$) for PEL 90% compared to 87% for EXT and GE digestion was influenced by treatment ($P = 0.03$) with PEL being higher than EXT. In conclusion, digestion and rate of intake was decreased by extrusion feed processing.

DEDICATION

For Dr. Josie A. Coverdale

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CHAPTER I

INTRODUCTION

The nutritional requirements of horses are based on the assumptions of how well the animal digests and absorbs the nutrients being fed. One factor that may influence these requirements is how the feedstuffs are processed. The level of feed processing can alter the digestibility of the feed by the gelatinization of starch and denaturation of protein contained within the concentrate (Rosenfeld 2009). One type of processing, known as extrusion, uses a pressurized, high-temperature steam, and allows the feedstuff to be exposed to intense mechanical shear forces (Singh et al., 2007). Extrusion allows for the denaturation of proteins, oxidation of lipids, and the alteration of carbohydrates that alters the nutritional quality of the feedstuff. Gaebe et al. (1998) demonstrated extruded corn and grain sorghum were more highly digestible to the animal with a digestibility DM for extruded being 77.10 compared to dry-rolled at 72.70. Therefore, this process has been shown to increase nutrient digestion in other species; however, research in this processing method in horses is very limited.

The level of processing will also impact the ability for glucose and other simple sugars to be digested in the small intestine. To look at the level of availability the glycemic index(GI) was introduced in humans and then was adopted into the horse world. There are three levels of a glycemic index that a feed can be: high (GI >70), medium (GI 55-69), and low (GI < 55). Rodiek et al. 2007 showed that sweet feed, corn, and oats were in the high glycemic index category. They also showed that grains were

typically greater in value than forages or by-products. There are multiple factors that can affect the GI of a feedstuff with one example of that being how the feedstuff is processed. Hoekstra et al. 1999 evaluated the difference between cracking, grinding, and steam processing of corn. They showed that steam flaking changed the glycemic index of the corn greater than the other two types of processing due to a higher plasma peak glucose concentration. Previous literature has focused on the glycemic responses to simple grain sources in addition to processing. However, limited information is available on formulated concentrates.

CHAPTER II

REVIEW OF LITERATURE

Nutrition

Equine Digestion Overview

Equines are classified as a monogastric or non-ruminant herbivore whose digestive tract is broken down into two sections. The first section is known as the foregut which is comprised of the mouth to the ileum and is responsible for enzymatic digestion. The second section is known as the hindgut that is made up of the cecum and two colons and is responsible for fermentative digestion.

Beginning with the mouth there is a mechanical change of the feed particles due to the animals chewing which for roughage can be between 3000-3500 movements/ kg. With the presence of food being chewed in the mouth there is also the production of saliva which can amount to about 35-40 L/d with little to no salivary α-amylase present (Hoffman, 2009). Feedstuff is then moved through the esophagus by peristalsis into the stomach which is only 10% of the total gastrointestinal tract and divided into two main regions of squamous (or non-glandular) and glandular region with the glandular being broken down into the fundic and pyloric regions. Feed stuff is first moved into the saccus caecus region which is the nonglandular region of the stomach where most bacterial fermentation that occurs in the stomach happens. This fermentation has a limited microbial population and mainly produces lactic acids from starch fermentation. The digesta then moves down into the fundic region where enzymatic digestion of protein and fat begins and then moves through the pyloric region, and out the pyloric

valve into the small intestine. From the stomach, the digesta moves to the small intestine which is the primary site of digestion and absorption for most nutrients and includes soluble carbohydrates, lipids, and proteins. The small intestine is approximately 30% of the total gastrointestinal tract and is divided into three portions; duodenum, jejunum, and ileum. Beginning with the duodenum in the proximal region there is an increase in pH, addition of both enzymes and buffer (bicarbonate) from the pancreases, and bile from the liver since horses do not have a gall bladder. When moving through the duodenum there is an increase in structure surface area and a decrease in digestive rate allowing for the absorption of nutrient in the distal region. In the jejunum, there is the continuation of absorption as digesta is moved through the intestine with finally moving to the ileum where it is the last chance for prececal absorption of nutrients. It is preferred that soluble carbohydrates, are digested pre-cecally, while allowing the fiber component of the diet to move into the hindgut for fermentation.

Therefore, whatever is not digested in the foregut, moves on to the hindgut or more specifically the cecum which is a blind pouch, small colon, and large colon which makes up 65% of the gastrointestinal tract of the horse. In the large intestine, there is only microbial fermentation and no enzyme or mucus secretion. The end products of the microbial fermentation are gas, volatile fatty acids (VFAs), microbial crude protein (MCP), vitamin K, and B vitamins. The main constituents that is associated with fermentation are soluble carbohydrates, however, if there is any non-structural carbohydrate, protein, and lipids that did not get fully digested in the small intestine they will also move into the hindgut for fermentation.

Carbohydrate Digestion

According to the NRC (2007), the total tract starch disappearance is greater than 90% but how it is digested pre-cecally is variable in measurements. This can be attributed to the abundance of amylase in the digestive secretions and the exposure that the amylase has on the starch, however relatively little is known about the factors effecting the digestive enzyme quantity in the gastrointestinal tract (NRC ,2007). Carbohydrates are the most common nutrient utilized by the horse for energy and can be classified into two categories relative to digestion.

The first are non-structural carbohydrates and are comprised of the cell content components like simple sugars and starch. Starch can be categorized into two types amylose and amylopectin. Amylose is comprised of glucose units and contain α- 1, 4 linkages which can be broken down by enzymatic digestion. Amylopectin also contains α- 1,4 linkages but every 20 units has a α- 1,6 branch and is more digestible than amylose due to its less rigid structure. These polysaccharides are broken down by pancreatic amylase which cleaves two glucose units from molecules containing five or more and turning them into disaccharides (lactose, maltose, isomaltose and sucrose), which are then activated by brush border enzymes to be broken down into monosaccharides. These brush border enzymes are produced by the enterocyte to make glucose, galactose, and fructose. Glucose and galactose move through transporter SGLT1 which is a Sodium glucose cotransporter type 1 carrier protein that transports them into the enterocyte, glucose is also able to utilize facilitated transport through glucose transporter 2 (GLUT 2), and fructose also utilizes facilitated transport through

glucose transporter 5 (GLUT 5). Once in the enterocyte fructose, glucose, and galactose move down the gradient and are transported by GLUT 2 out of the enterocyte (Hoffman, 2009). Galactose is converted to glucose mainly in the liver, this glucose can be used as an energy source and can be stored as glycogen. Glucose can also be released into the blood stream to allow for tissues to utilize glucose. This transport of glucose into tissues can be done by GLUT 3, and muscle and adipose GLUT 4.

The second are structural carbohydrates or dietary fiber (DF) which cannot be hydrolyzed by digestive enzymes in monogastrics (Montagne et al. 2003). These are cell wall components made up of lignin, hemicellulose, and cellulose, and measured as NDF and ADF values in the Van Soest system. The components except for lignin get fermented by microbial amylase and cellulase produced by cellulolytic and amylolytic bacteria to produce volatile fatty acids (VFAs), B- vitamins, Vitamin K, and microbial crude protein (Hoffman, 2009). VFAs also provide another energy source to the animal and can provide up to 30% of the horse's energy requirements. Acetate is used as an energy substrate for muscle tissue, propionate is utilized to produce glucose via gluconeogenesis which is then used for glycogen, aerobic/ anaerobic energy, fat synthesis, and muscle/liver glycogen (Montagne et al. 2003).

Protein Digestion

Protein is a major component of body tissue, considered 16%N, made up of amino acid and is used to synthesize tissues, hormones, antibodies, and enzymes in the body. The goal of the digestion of protein is to denature or unfold the protein structures into primary proteins. Digestion begins in the stomach by utilizing hydrochloric acid

(HCL) and pepsin producing a combination of protein, peptides, and free amino acids. HCL denatures protein structure into primary structures and activates pepsinogen into pepsin. Pepsin is an endopeptidase and acts on these primary structures of protein by performing a rough chop to break down some of the protein into peptides and amino acids before they are moved into the small intestine. Once moved into the small intestine these products from the stomach are subjected to pancreatic zymogens: trypsin, chymotrypsin, and carboxypeptidase after they are activated by trypsin which is first activated by enteropeptidase which is produced by the brush border. These pancreatic zymogens are both endopeptidases (specific cleavage) and exopeptidases (non-specific cleavage) and are used to prevent auto digestion in the small intestine. The end products of protein digestion in the small intestine are amino acids, dipeptides, and tripeptides.

Absorption of these end products occurs along the length of the small intestine but mostly in the distal duodenum and proximal jejunum. Amino acids (AA) are transported into the enterocyte by specific transporters and basolateral AA transporters while peptides are moved into the enterocyte by peptide transporter (PEPT1) that requires a co-movement of protons. Absorbed amino acids can go to tissue protein synthesis, hormone/ enzyme/ other metabolite synthesis, and deamination or transamination in the liver. For something to be a good protein source it must be prececally digested due to pre-cecal absorption and it is important to acknowledge that horses do not have a crude protein requirement but instead an amino acid requirement (NRC, 2007). If protein is digested in the hindgut it gets exposed to fermentation where some of the nitrogen is utilized to create microbial crude protein, there is minimal AA

absorption, and ammonia is produced. Th ammonia produced is utilized for microbial protein synthesis which is not available to the horse since it is produced after the site of absorption (Rosenfeld and Austbo 2009). It is important to note that a horse's protein requirements can be met but their digestible energy (DE) to crude protein (CP) ratio may be deficient as well as the DE: CP may be met and the AA requirement may not be.

Lipid Digestion

Dietary lipids are largely made up of fatty acids and triacylglycerides, are the secondary source of energy for horses, and are more reduced than carbohydrates (2.25 x energy) (NRC, 2007). There are three fatty acids required by horses; linoleic (omega 6), linolenic (omega 3), and arachidonic acid. It is interesting to note that omega 3 fatty acids are better than omega 6 fatty acids. However, it is harder to give horses omega 3 fatty acids due to the best source of an omega 3 being fish oil which is not very palatable to horses. Digestion of lipids occurs primarily in the small intestine and utilizes the assistance of bile from the liver for lipid emulsification and pancreatic lipase to break down lipids into 2-monoacylglycerols, lysolecithin, cholesterol, fatty acids, and bile salts. Emulsification is both chemical and physical and done to increase digestion by increasing the surface area for the enzymes. These are combined to form a micelle due to all the components being hydrophilic and needing to be moved through digesta for the micelle to empty its contents into the enterocyte. Once they are in the enterocyte the glycerol and short chain fatty acids move into mesenteric blood stream, 2-monoacylglycerol and free fatty acids reform triglycerides (which then add protein and CHO to form chylomicron), and the chylomicron is produced and enters blood through

thoracic duct. A chylomicron is a completed fat droplet that is transported to the cell membrane and exocytose into lymphatic circulation and slowly doles lipids out into the blood circulation with the remnant moving to the liver via endocytosis.

Blood lipids are transported by lipoproteins called chylomicrons, VLDL, LDL, and HDL, with the last three being produced from the liver. These lipoproteins undergo intravascular hydrolysis by lipoprotein lipase and are synthesized by myocytes and adipocytes. Fatty acids are transported from blood circulation by a complex with albumin which form a NEFA (non-esterified fatty acid) and area good measurement of total blood content. These NEFAs can be oxidized by the muscle and liver to be used as an energy source.

Energy Partitioning

We have previously discussed the three nutrients contributing to energy production in horses. A horse's energy requirement is represented through DE which is a representation of apparent digestibility and only accounts for the fecal energy loss of the animal. To look at the digestion coefficients in horse's digestibility trials must be conducted. These trials are performed based on Cochran and Galyean (1994) recommendations on how to look at digestibility. In horses and most other livestock species the greatest energy loss is the fecal energy loss. This fecal energy loss is composed of the undigested feed and metabolic products or endogenous sources. According to Cochran and Galyean (1994) the closest way to look at fecal energy loss is to look at the organic matter and dry matter digestibility due to the close relationship between them. This is one of the reasons a total fecal collection is utilized to go from

gross energy to digestible energy as well as look at overall total tract apparent digestion of different nutrients. Other methods that can be looked at when it comes to digestibility is prececal digestion. To look at prececal digestion cannulation, glycemic index, and mobile bag technique can be utilized. These digestion coefficients can be altered by feed processing techniques on the different feed components.

Dietary Management

Horse management is dependent on diets, snacks, housing conditions, and their daily routines. It is important to provide nutrients in a balanced ration that meet NRC requirements for the horse. A horse at maintenance can be maintained on a high-quality forage at a minimum of 1% BW/d. When the horse's requirements are greater than those met by the forage the animal is consuming concentrate is introduced into the diet. An example of when a time requirements get to high are in performance horses. When feeding concentrate it is important to consider the small stomach size of the horse and to avoid starch overload in the small intestine. When feeding concentrate the individual meal should not exceed 0.5% BW/meal and meals should be fed in several small meals to mimic the natural behavior of the horse while also decreasing passage rate and stimulating digestion (van Weyenberg et al. 2007). Rosenfeld et al. (2006) observed that both hay and processed grain have similar retention time in the gastrointestinal tract. Starch should be fed between 0.2-0.4% BW/meal to decrease chance of starch reaching the hindgut and causing possible incidence of colic and laminitis in horses.

Feed Processing

Processing of feed can influence the chemical, physical, and microbiological properties of feedstuff which can in turn improve the animal performance (NRC, 2007). These improvements can be done through methods such as dry rolling, cracking, steam flaking, pelleting, and extrusion. Grains are processed prior to feeding to enhance the digestibility and alter the site of digestion. This allows feed producers to get the best value of the grain since the grain can be matched to the digestive capacity of the animal and allows for the ability to alter the rate of nutrient release in the intestinal tract (Rowe et al., 1999). A primary benefit of feed processing is the effect on starch digestibility.

Grain characteristics that can affect the digestion of starch include seed coat, endosperm, non-starch polysaccharide (NSP), protein matrix composition, and starch gelatinization. The seed coat must be disrupted by either cracking or mechanical processing to expose the endosperm and allow for starch digestion. The nutritional content of the seed coat is of importance in its nutritional significance, for example, an oat grain hull makes up 25% of the dry matter and can be made up of high levels of lignin. The endosperm is important because it houses the starch granules which are then surrounded by a matrix made up of protein and NSP which in turn have a massive effect on the physical characteristics of the endosperm and play an important role in starch digestion. NSP is a big cause in the variation of cereal grain nutritional value in monogastrics because it can cause the digesta to increase in viscosity which can reduce digestion (Rowe et al., 1999). With processing, there is a gelatinization of starch which

causes the matrix binding to be interrupted and the starch cells are expanded at elevated temperatures.

The actual site of nutrient digestion is more important when looking at the benefit that feed processing has because pre-ceceal and hindgut digestion are very different from one another and need to both be considered. Under processing feeds can leave levels of anti-nutritional factors like trypsin in soy bean meal however, excessive heating may also reduce the availability of essential amino acids due to the presence of Millard reactions (NRC, 2007). One thing to consider is there are factors that can influence these results such as horse differences, grain type, starch level, and DM intake. The common methods utilized in the equine industry are chopping, cubing, wafering, and pelleting rations.

The main form of feed processing used in commercial equine feeds today is the pelleting processing. A pellet is an agglomerated feed that is formed from compacting smaller particles and utilizing a mechanical process of forcing the formed feed through a die opening in combination with moisture, heat, and pressure. Benefits to using this method are that it has been shown to decrease dust, increase palatability and thus increases voluntary intake, eliminate sorting, and allows for partial gelatinization of the starch molecule. Svinus et al. (2005) observed that with pelleting between 10-200g of starch/kg was usually gelatinized during the process.

Extrusion Feed Processing

The Extrusion Process

The principle aim of the extrusion feed processing is to achieve a high level of starch gelatinization while also disrupting the grain structure which is done by utilizing the effects of moisture, high temperatures, and pressure and has been shown to maximize beneficial effects while also minimizing detrimental ones. The general process is that feed is taken from the holding bin, through a mixing cylinder, fed into the extruder barrel, and once released from the barrel expansion happens as steam is released due to the sudden drop in pressure. An extruder barrel consists of a flighted Archimedes screw that rotates in the tight barrel and can be a single screw or a twin screw depending on the specific protocol being followed. Before feed ingredients are added to the barrel they are pre-ground and then as they work their way through the barrel they are exposed to pressure due to different sizes of the dies or the small openings at the end of the barrel that the feed is pushed through which exposes it to shear force. The barrel may have steam that is injected into it or can be steam-jacketed and can utilize temperatures from 125-170°C but the ingredients are only exposed to these temperatures for a short time (15-30s) which is called High temperature/ Short time (HTST). During this method, there are many parameters that must be controlled such as rate of ingredient flow into extruder, temperature of barrel, and size of die orifices. (Serrano, 1997).

Effect on Feed Constituents

Extrusion processing involves changing or altering the digestibility and utilization of nutrients such as altering the carbohydrate structure, denaturing proteins,

and oxidation of lipids. When looking at the starch molecule there is usually a complete gelatinization occurring at temperatures greater than 120°C, percent moisture between 20-30, and high shear force (Cheftel, 1986). The gelatinization is the breakdown of intermolecular bonds in the presence of water and heat that allows for the bonding sites to engage more water essentially dissolving the starch granule in water which allows for the improved facilitation of enzymatic digestion of starch in the body. Both gelatinization and the impact that the feed processing has on the utilization of starch in the body is dependent on the specific extrusion process. Rosenfeld and Austbo (2009) observed that extrusion increased the total tract digestion of starch to 0.978 compared to pelleting of 0.958. To severe of a process however, can cause retrograded starch to form. Retrogradation of starch is the crystallization of gelatinized starch in amorphous matrix and involves the formation and subsequent aggregation of double helics of amylose and amylopectin (Svinus et al., 2005). This agrees with the thought that too less or severe of a process may cause the molecules to not be utilized and that moderate/mild extrusion processing may be the proper way to improve the nutritional quality (Masatcioglu et al., 2014).

Protein undergoes a structural unfolding and aggregation when nutrients are exposed to the moisture and shear force during the extrusion process. Extrusion of soybeans and oilseeds have shown an example of improved digestibility of protein and bioavailability of the Sulfur amino acids. As seen with the starch it is recommended to use a mild extrusion process to enhance the digestibility of these proteins. Rosenfeld and Austbo (2009) observed that with extrusion there was an increase in total tract digestion

when compared to pelleting with values of .818 and .798 respectively. However, with protein there can be some problems that are encountered such as cross-linking which can reduce the functionality of the protein, racemization or changing L-amino acids into D-amino acids, oxidation of sulfur containing amino acids, and loss of lysine due to Millard reactions which are a non-enzymatic browning and flavoring reaction that favor high temperatures and low moisture environments. This reaction has been shown to affect the amount of lysine available to the body from feed by binding lysine to a ε-amino group for up to 61.8% of lysine in pet food. In a study done by van Rooijen et al. (2013) observed that after extrusion the amount of lysine was reduced for wheat to 2.8% and dehulled rice to 8.6% however, they also saw an increase in lysine for barley (4.8%) and maize (9.5%).

Extrusion causes an inactivation of lipase and lipoxidase which helps protect against oxidation during storage by exposing these lipids to higher temperatures and reducing the lipase activity and moisture level therefore, decreasing the free fatty acid development. There is also the possibility of the formation of lipid-starch and lipid-protein complexes. These starch-lipid complexes are negatively associated with the extent of swelling of the nugget due to an increase in hydrophobicity. Complexes may impair digestion both directly due to the the need for water with enzymatic digestion and indirectly due to the lower extent of gelatinization in processing (Svinus et al., 2005).

Increased mineral absorption has been observed after the extrusion process in a study done by Alonso et al. (2001) which was probably due to the destruction of polyphenols during the heat treatment. Tran et al. (2008) observed a decrease in

manufacturing of pet food because of the extrusion process such as a 20-65% loss of vitamin A. They also saw that apparent absorption of iron, copper, and phosphorous from extruded diets was greater than the other processing methods. With the effect on extrusion to vitamins and minerals it is noted that the decisive factor for increasing the availability of these is due to the treatment during extrusion than the other processing components (Tran et al., 2008).

Benefits

Alonso et al., 2001 showed that a benefit derived from the extrusion process was the partial or destruction of antinutritional factors such as protease inhibitors, hemagglutinins, tannins, phytates, and trypsin inhibitors; all of which can have crippling effects on the nutrient utilization in the animal's digestive tract. With regards to trypsin inhibitors there is evidence to suggest seeing an increase in inactivation of trypsin inhibitors and growth retarding factors such as lectin as well as a decrease in activity for chymotrypsin inhibitors, and α-amylase inhibitors (Serrano, 1997). Tran et al. (2008) showed that a barrel temperature range between 133-139°C was enough to inactivate 95% or more of trypsin inhibitors. Tannins may form what are known as insoluble complexes with divalent ions in the gastrointestinal tract which causes a lowering of their bioavailability, rather they cause plant proteins to be difficult to digest for the animal.

Finally, using this extrusion method has also shown to increase shelf-life and safety by the thermal destruction of viable spores and any bacterial contamination that might be present in the feedstuff. Allowing for the owner to buy in bulk if needed to

possibly save resources and not need to worry about the feed going bad and losing money (Van Rooijen et al., 2013).

Use in Other Species

Extrusion feed processing has been shown to increase total tract apparent digestibility of fat, nitrogen retention, and essential amino acids (except lysine and histidine) in laying hens as well as show a significant increase in ileal digestibility of crude protein and amino acids in chickens (El-Khalek and Janessens 2010). This increase in digestion was again seen by Gonzalez-Alvarado et al. (2007) who saw an increase in total tract apparent retention of most nutrients in corn diets.

In swine, the process has been shown to increase the proportion of rapidly digestible starch (RDS) with a reduction of the proportion of slowly digestible starch (SDS) and resistant starch (RS). This was seen due the gelatinization of the starch molecule and mechanical rupture of the plant cell wall which allowed the enhancement of surface contact between the substrate and its digestive enzymes leaving the starch almost completely digested by the small intestine leaving very little to be fermented by the colon (Sun et al., 2006). Heat processing of the cereal grains has been a common practice in piglet diets to allow for the improved nutrient availability to the body and productivity performance due to the major impact it has on site and extent of nutrient absorption in the gut of pigs. Sun et al. (2006) also reported an increase in the digestion coefficients of DM, OM, and CP of a pea and potato starch/wheat bran diet as well as the improvement in ileal digestion of starch in winter pea diets.

In a study in finishing steers there was an increase in apparent DM and starch digestibility in those steers who were given the extruded grains instead of the rolled grains specifically, for starch 96.4% compared to 85.5% respectably. However, NDF, ADF, dry matter intake, digestible dry matter intake, and digestible energy intake per unit of body weight wad lower for the steers receiving the extruded grain (Gaebe et al., 1998). Moving into dairy cattle there was an increase in postruminal digestibility of the nonstructural carbohydrates (NSC) as well as an increase in rate of ruminal degradation for both organic matter and NSC. Shabi et al. (1999) also showed that the extrusion process decreased the density of the corn by 37.5%, that total tract NDF digestibility was increased, and that ruminal ammonia nitrogen and plasma urea nitrogen were decreased by 13% and 30% when cows were fed extruded diets.

The fish community also uses the process of extrusion for their feed. It has been shown to increase the apparent digestibility of dry matter, crude fat, and gross energy. This process has also been shown to decrease the apparent digestibility of CP, phosphorous, copper, iron, and zinc in trout (Cheng and Hardy ,2003). The increase in the DM, crude fat, and gross energy allows for a positive effect on the environment due to reduction in the excretion of solids into the water by the fish as well as an improved feed conversion rate by the trout. Hilton et al. (1981) showed that the extrusion pellets were more durable, had superior water stability, and absorbed more water than the steam pellets. Due to the processing method, the bioavailability of the carbohydrates for the trout was improved as well as the feed efficiency and liver glycogen levels.

Dog and cat food have been big supporters in the use of extrusion feed processing as 95% of the feed is processed this way and allows for the increase in digestibility of raw ingredients as well as the expansion, dehydration, and shaping of the kibbles. The versatility of this technology allows to mix diets and functionally improve, detoxify, sterilize, and texturize a variety of food commodities and ingredients. Feeding an extruded diet showed an increase in the improvement of digestibility of crude protein in growing dogs (Tran et al., 2008) Extrusion has been shown to have the largest effect on reducing levels of several enzyme inhibitors and lectins play a major role in affecting palatability by controlling the level of specific mechanical energy used. The main effects of extrusion seen in pet food today are starch gelatinization, protein denaturation, vitamin loss, and inactivation of nutritionally active factors (Tran et al., 2008).

Use in Equines

In horses, it has been shown that extrusion feed processing increased the preceal digestibility of starch (Julliand et al., 2005) McLean et al. (2000) observed a decrease in the total DMI in the extruded feed fed to ponies compared to rolled and micro ionized feed (3.53, 3.67, and 3.57, respectively). However, most of the data on extrusion processing effects in horses has been looked at for the glycemic response from the horse and there is limited data in how the extrusion feed processing affects apparent digestion in horses.

Glycemic Index

General Overview

The glycemic index (GI) was introduced to classify multiple sources of carbohydrates based on their effect on blood glucose post meal which is compared to a standard feed value by taking the quantities of carbohydrate and providing a measure of quality but not quantity produced. (Vervuert and Coenen 2005) FAO/WHO, 1998 defined the term as the area under the blood glucose response curve of a 50g carbohydrate portion of a test food, that is expressed as a percentage of the blood glucose response curve of the same amount of carbohydrate from the standard consumed by the same subject. There are three different classes on the glycemic index system to classify a food or feedstuff: high (GI >70, high amount of NSC concentration), medium (GI 55-69), and low (<55); low GI carbohydrate food means it is digested and absorbed slowly where as high GI carbohydrate shows rapid digestion and absorption. When expressing a value for a glycemic index it given as a percentage of the glucose response to a standard food (in humans this is usually white bread). (Rodiek and Stull 2007)

When looking at the glycemic index one also wants to look at the insulin response that is associated with the glucose response being seen. When there is a feed with a high glycemic index number it usually means that is has a higher insulin response curve to go along with it. (Ralston 2007) When there is an increase in insulin levels it causes an increase in glucose uptake by tissues such as muscle and adipose by activating the GLUT 4 glucose transporter which is insulin dependent.

There has been a high correlation between the rate of glucose release from starchy foods in vitro using pancreatic brushy border enzymes, and the glycemic response in vivo. (Brouns et al. 2005) With these findings and the fact that indirect measures of prececael starch digestion support results from digestion trials allow the utilization of the glycemic index to interpret indirect prececael digestion of different carbohydrate feedstuff. However, there are some factors that can cause variation in the values of a glycemic index. These factors are starch and sugar content, food composition, cooking or processing, and rate of eating. With food composition the presence of fat, protein, anti-nutrients, and acidic compounds can alter the glycemic index values of a food. Variations can also occur when looking at one particular feedstuff compared to a mixed meal component. A glucose and insulin response to a meal is not usually the same as a glucose and insulin response to each individual component of that meal. This means that if you took each component and used those to calculate the response for the whole meal if you compared that to the actual response of the whole meal the numbers will not always match up due to interactions between the components of the meal being ingested at the same time. (Brouns et al. 2005)

Processing Effects

The effect of feed processing on glycemic index values in humans or horses can change for the same feed stuff that was just processed differently. The big component in this is the fact that the structure of the starch changes with processing. The granular structure of starch can be disrupted by mechanical processing or processing involving heat and pressure in combination with moisture. (Vervuert and Coenen 2005) This

causes a change in values since glycemic index is an indirect measurement of prececael starch digestion however, Verveurt an Coenen 2005, found that the effect of grain processing on the glycemic index was due to the amount of starch intake the subjects obtained. The matrix structure of starch has an influence on the glycemic index in horses which was seen when looking at two rations that had a similar starch content but were processed differently (textured sweet feed vs. pelleted feed) and saw two different glycemic responses. (Harbor et al. 2003)

A study done by Gordon et al. 2008 looked at the glycemic index response with pelleted and extruded processing methods. They saw that the pelleted feed showed a longer time for peak glucose, lowest average insulin concentration, lowest average glucose response, and higher rate of ingestion of feed than extruded. A positive correlation was seen for both feed forms when looking at consumption time and time to peak insulin but not for peak glucose.

Human Application

The glycemic index was originally developed as a means of comparing carbohydrate sources and their potential for raising the blood glucose levels to aid in diet formulation for diabetic people. Diets high in GI are associated with an increased risk in insulin resistance, dyslipidemia, and cardiovascular disease. There has been evidence seen that if you increase the amount of carbohydrate intake that has a low glycemic index then there is pancreatic β-cell function in subjects that have impaired glucose tolerance. Which is important since the pancreatic β-cells are responsible for storage and release of insulin therefore, the function seen shows that the subjects can produce and

release insulin in response to the low GI food being consumed. (Vervuert and Coenen 2005) It has been theorized that if a person reduces their daily glycemic load then they may be at a reduced risk for developing diabetes and cardiovascular disease.

There are a couple considerations to the methodology of the glycemic index in humans. Tests should be performed in the mornings due to the stability of the fasted condition and ideally this means before 10 am and after the person has been fasted for 10- 14 hours. The reason for this is that there can be individual differences if the time of day is different as well as possible meal influences from meals prior to the fast. The fasts are required because there is a change in falling plasma insulin and increasing lipolysis, if you do not fast then the pervious meal will skew the results of the food that is trying to be tested. Another thing to consider is the time it takes to eat the carbohydrate food or glucose solution being tested. Rodiek and Stull 2007, showed that the rate of consumption of the solution radically effected peak glucose concentration and AUC even though the total amount of glucose intake was the same between both people.

Equine Application

The metabolism of starch, sugar, and glucose in the horse is like that of a human as well as having some of the same health complications associated with it such as obesity and insulin resistance as well as, laminitis, osteochondritis, and polysaccharide storage myopathy on the horse side. Therefore, people want to take the glycemic index from the human side of nutrition and apply it to horse nutrition. However, there are two differences that need to be considered. First the fact that there may be some carbohydrate fermentation in the stomach of the horse. The extent of this fermentation varies

depending on the individual animal's gastric flora, nature of the feed being consumed, and the gastric emptying rate that particular animal has. Second is that there is a risk of digestive disturbances in the consuming a meal with large amounts of starch/ sugar in them. Thus, a high glycemic index feed may result in a short-term problem that is not associated with any long term metabolic influence of that feed. (Harris and Geor 2009) The benefit to creating a glycemic index of the common horse feeds to categorize them as either high or low glycemic index feeds would be that the industry would be able to formulate feeds to produce certain glycemic and insulinemic responses to help horses either avoid or ameliorate metabolic issues such as insulin resistance. Zeyner et al. 2006 showed that there is a close relationship between the glycemic index and insulinemic index response when looking at adult quarter horse data. He saw that when the feed had the high glycemic index value it also had the high insulinemic value to go along with it and vice versa. The glycemic index system could also be used by the performance industry to produce elevated glucose levels to provide powerful energy sources such as when carbohydrate oxidation gives a large percentage of the animal's energy requirements.

The actual protocol for performing a glycemic index trial in horses has been variable, making it very difficult to compare results across multiple studies. For example, in one study the ingredients, nutrient composition, amount/ origin of starch, and texture of feed were variable causing the differences in values to be inconclusive since they could not attribute the differences to a single factor. Some other variable factors to consider are meal size, enzymatically digestible starch amount, amount of

fiber and fat, processing method, rate of intake for the individual animals, and number of actual calories offered (Mcal DE). It is known based on looking at the trend of studies that cereal grains and molasses have higher glycemic index values than forages. This is most likely the reason that Pagan and Harris (1999) saw a decrease in glycemic index value when they fed hay along with concentrate when comparing the values to the concentrate alone. When looking at insulin response there are a couple of things that could possibly alter this such as the release of gastric inhibitory polypeptide (GIP) from the small intestine and the dietary content of carbohydrates, amino acids, and fats in the feed. In a study done by Rodiek and Stull 2007 they found values of 128, 113, 105, 100, 24, 21, 13, 81, 63, and 7 for sweet feed, corn, jockey oats, oats (the standard), beet pulp, alfalfa, rice bran, barley, wheat bran, and soy hulls. There was not a correlation between digestible energy (DE) or carbohydrate composition to the glycemic index values that would have allowed them to rank the feeds. There was however a significance between glycemic index and non-fiber carbohydrate, non-structural carbohydrate, starch compositions, and intake of the diet. The plasma peak in glucose for this study happened at about 90 – 120 min. for all feeds and all concentrations returned to baseline values by the end of the 300 min. period except for the oats. They also saw a trend showing that the index values were greater for grains than forages as well as they had a significant horse effect on the glycemic index values. No standardized index has been formed and not a lot is known about the effects of feed processing and mixed meals on the glucose and insulin responses. It has been shown that when comparing steam flaked corn to cracked corn the steam flaked had a greater glycemic index value and that pelleted feed

produced a greater response than non-pelleted. (Harbour et al. 2003) When bringing this to the horse side some factors that need to be considered are: age, breed, physiological state of the animal, and the form of the feed. This is due the fact that for the index to be reliable it must account for the differences among feeds fed separately and for when they are fed together like in a commercial diet. (NRC 2007)

The influence that this could have on the industry can be seen in two different ways. First there is the influence on the health aspect of the industry. One of the big reasons for utilizing the glycemic index is that there is an assumption of close correlation between the postprandial blood glucose response and insulin regulation which can be used to help control insulin resistance in horses. (Kronfeld et al. 2005) Polysaccharide storage myopathy (PSSM) has also been thought to benefit from the utilization of a glycemic index. PSSM shows signs of stiffness and cramping in varying severity during attacks that is characterized by an extremely high accumulation of glycogen which is caused by an increase in synthesis and deposition of abnormal polysaccharides in skeletal muscle fibers. It is thought that the promotion of PSSM is from an enhanced insulin sensitivity and increased glucose uptake with elevated muscle glycogen synthesis since these horses show an abnormally high insulin sensitivity. It is being speculated that feeding a low glycemic index feed would benefit these horses and possibly prevent future episodes from occurring. (Zeyner et al. 2006) A glycemic index would be beneficial to the geriatric population who are glucose intolerant and could benefit from being fed a low glycemic index feed to influence the dietary glycemic load of the animals. (Vervuert & Coenen 2005) Secondly the influence on the performance horse

industry. A drop in the glucose concentrations in a performance horse can indicate that there is a lack of availability for the muscle or brain which can in turn cause bad effects on the performance of the animal, thus, a feed with a high carbohydrate content and low glycemic index would benefit the animal especially since blood glucose homeostasis is important in offsetting an endurance horses fatigue. In a performance horse muscle glycogen replenishment is needed after a bout of exercise and this replenishment is determined by the availability of the substrate (mostly glucose and some lipid metabolites), this substrate availability is the principle limiting factor in muscular performance. Lacombe et al. 2006 showed that horses who were fed a high glycemic index diet had a 40-50% higher rate of glycogenesis than those fed the lower glycemic index feed and that muscle glycogen synthesis was greater as well when looked at over 72 hours of recovery. This study showed that horses going through heavy exercise regularly could benefit from being fed a high glycemic meal. There can be multiple applications to the use of a glycemic index to the horse industry however, there needs to be a standard way to obtain the data for both individual feedstuff but also mixed commercial rations.

Conclusion

The benefits of extrusion feed processing are evident in multiple livestock species. The ability of this processing method to improve apparent digestion coefficients has been effectively demonstrated in both non-ruminants and ruminants. However, the effect of extrusion feed processing on apparent digestion in horses has yet to be determined. Therefore, the objectives of the current study were to determine the effect of

extrusion feed processing on nutrient utilization in mature geldings and the glycemic index response between processing methods.

CHAPTER III

MATERIALS AND METHODS

Horses and Dietary Treatments

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University. (AUP# 2016-0304)

Nineteen mature Quarter horse geldings (466 to 697 kg BW; 9 to 18 yr age) were used in a completely randomized design. Prior to the start of the study horses were stratified by age and BW and randomly assigned to one of two treatments: a pelleted diet (PEL; n = 10) or an extruded diet (EXT; n = 9, Life Plus, Muenster Milling, LLC). Dietary treatments were formulated to be isocaloric and isonitrogenous and were fed at 0.5% BW (as-fed) daily, and all horses received 1.5% BW coastal Bermudagrass (*Cynodon dactylon*) hay (as-fed) daily (Table 1). Horses were fed at 0630 and 1830 h daily and were allowed 3 h to consume their meal. Orts were collected and weighed. Horse ate an average of 1.47 kg/d of concentrate and 4.39 kg/d of hay throughout the experiment. The experiment was divided into 4 phases: 1) 7-d transition to dietary treatments, 2) 14-d adaptation to dietary treatments, 3) 4-d total fecal collection, and 4) 1-d glycemic index study. During the first two phases horses were fed in individual 3 × 3 m stalls and allowed to roam on a 9 m × 600 m long dirt infinity track after the feeding period of 3-h.

Table 1. Nutrient composition of diets and forage used in the experiment.

% DM basis	PEL[1]	EXT[2]	Forage[3]
CP	15.43	14.36	7.56
NDF	28.69	33.47	63.55
ADF	20.52	22.97	33.88
Starch	21.17	20.00	2.26
Ca	1.3	1.38	0.43
P	0.68	0.75	0.12

[1]PEL = pelleted concentrate

[2]EXT= extruded concentrate

[3]Forage= coastal Bermudagrass hay

Sample Collection

Intake and digestion observations were made on d 22 through 25. Feed, hay, and ort samples were collected on d 21 through 24 to correspond with fecal samples collected on d 22 through 25. Feed and hay were sampled as it was being fed by obtaining 400 g of hay and 400 g of each dietary treatment daily. Following removal orts were weighed and approximately 200 g were retained for analysis. Fecal bags (Bun-Bag, Inc., Sagle, ID) were placed on geldings to collect feces over a 24-h period. Bags were removed and emptied at 0630, 1230, 1830, and 0030 h daily. The feces collected over each 6 h sampling period was homogenized and a sample was taken (3% of total fecal matter) and frozen at -20°C. During the 24-h fecal collection horses were housed in the same 3 × 3 m stalls that they were fed for 23-h a day. Each horse received a 1 h turnout time with no fecal harness on. During this turnout time the feces was collected in a separate bag so that the weigh could be added to the total fecal amount for the

appropriate 6-h sampling period. After the last sample had been taken the horses will returned to being fed at 0630 and 1830 h daily and turned out on the infinity track for day 26 and 27 of the project to allow for two-days of recovery before the glycemic index.

Rate of intake for each animal was observed in the morning feeding on d 17 through 21. Each animal was given 5-m to eat as much of their pre-weighed feed as they could. After the 5 min were up feed was collected, weighed back, and then given back to the animal to finish their meal. Calculations for rate of intake were then made off of these measurements over the 5-d period.

For the glycemic index on d 28 horses were placed into feeding stalls and catheterized one hour before the start of the glycemic index. The jugular catheter site was prepared by clipping the coat to a sanitary length (blade size 40), and sterilized using Chloradine scrub 4% in addition to isopropyl alcohol 70%. Lidocaine was used as a local anesthetic and injected subcutaneously at the site of catheter insertion. Catheters were placed and a 30 inch extension set was attached to the catheter and secured with Elasticon. All horses were allowed to acclimate for a minimum of 1-h after catheters were placed and prior to sampling. Glucose samples were taken at 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, and 480 minutes after feeding and Insulin samples at 0, 60, 120, 180, 240, 360, 480 minutes after feeding. Approximately 10 mL of whole blood was collected via indwelling jugular catheter into evacuated tubes containing heparin. The whole blood was centrifuged at 2000 x g for 7 minutes. The plasma was

collected and stored in 5 mL transfer tubes at -20°C until further analysis. Throughout the glycemic index the horses were allowed ad libitum access to both forage and water.

Sample Analysis

Grain, hay, ort, and fecal samples were dried at 55°C in a forced-air oven for 96-h, allowed to equilibrate for 24-h, and weighed for determination of partial DM. Sampled were ground in a Wiley mill to pass a 1 mm screen. Hay and feed samples collected during the period were pooled across days on an equal weight basis. Ort samples were composited in proportion to their daily refusal by horse across days, while fecal samples were composited by gelding across days. Hay, grain, fecal, and ort samples were dried at 105°C for 24-h to determine DM. OM was determined by the loss in dry weight during combustion for 8 h at 450°C. NDF and ADF analysis was performed on feed, hay, ort , and fecal samples using the Ankom Fiber Analyzer with sodium sulfite and α- amylase admitted and without correction for residual ash (Ankom Technology Corp., Macedon, NY). Direct calorimetry using a Parr 6300 Calorimeter (Parr Instrument Company, Moline, IL) was utilized to measure gross heat of hay, feed, ort, and fecal samples. Digestibility of energy was calculated as the remainder of consumed minus fecal excreted energy expressed as a percentage of intake. Total tract digestion coefficients for DM, OM, NDF, and ADF were determined using total collection, as described by Cochran and Galyean (1994).

A commercial glucose colorimetric assay kit (Cell Biolabs, Inc., San Diego, CA) was utilized to evaluate the plasma glucose concentrations in the blood. Samples were analyzed in duplicate. Test plates were run in order to determine the correct dilution

needed for sample analysis. Plasma was prepared for analysis by allowing to completely thaw and centrifuging at 10,000 rpm for 5-m then applying a dilution factor of 1:250 with assay buffer. Standards were prepared based on the protocol provided. After standards and samples were prepared 50 µL of each was added to the appropriate wells on a 96 well plate based on the plate layout made beforehand. To these standards and samples 50 µL of a reaction mix was added to each of the wells. The plate was then tapped on the side to ensure complete mixing, sealed with a plate cover and foil to protect it from light, and allowed to incubate at 37° C for 15-m. Once the incubation was over the optical density at 540nm was read using a microplate reader (Biotek, Winooski, VT) and concentrations were produced from comparing the OD values of the samples to the standard curve.

Plasma insulin concentrations were determined by an equine ELISA kit (Mercodia Inc., Winston Salem, NC) which is a two-site enzyme immunoassay. Calibrators for this kit came ready to go and plasma samples did not have to be diluted for this kit. To begin each 96-well plate layout was planned and from there 25 µL of each calibrator and sample were added to the appropriate wells. To these wells 100 µL of enzyme conjugate was added to each well. Plates were then put on a plate shaker (VWR, Radnor, PA) for a 2 h incubation at room temperature (18-25°C) at 700 rpm. Plates were then washed 6 times with 700 µL of buffer 1X solution per well using an automatic plate washer (Biotek, Winooski, VT) after the last wash the plate was inverted and taped firmly against absorbent paper. To the plate 200 µL of Substrate TMB was added to each well and then incubated on the bench for 15-m at room temperature (18-

25°C). 50 µL of stop solution was then added to each well and plate was put on plate shaker for about 5 seconds to ensure mixing of solution. Optical density at 450nm obtained using a microplate reader (Biotek, Winooski, VT) and used to calculate concentrations.

Statistical Analysis

Intake, digestion, and rate of intake were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Term in the model was treatment and LSMEANS was used to calculate treatment means. Area under the curve (AUC) for glucose and insulin concentrations was determined using PROC EXPAND procedure of SAS (SAS Inst. Inc., Cary, NC). All AUC data and peak concentrations of glucose and insulin, as well as foal physical growth measurements were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Main effects tested were treatment, time, and treatment by time interaction. A paired t-test was used to compare differences between dietary treatments. Means are reported as LSMeans ± SD. Statistical significance was determined as $P \leq 0.05$ and trends toward significance were determined as $P \leq 0.10$.

CHAPTER IV

RESULTS AND DISCUSSION

Intake Parameters

Dietary treatment affected concentrate DM intake, concentrate OM intake, and rate of intake (Table 2). There were no effects on total DM, total OM, NDF, ADF, GE and DE intake.

Table 2. Least square treatment means of intake parameters including forage DM, concentrate DM, forage OM, concentrate OM, DM, OM, NDF, ADF, Starch, DDM, GE, DE, and rate of intake in horses receiving a pellet concentrate (PEL) and a extruded nugget (EXT).

	Treatment			
Item	PEL	EXT	SEM	*P*-value
No. of observations	10	9		
Intake, %BW				
Forage DM	1.14	1.12	0.04	0.70
Concentrate DM	0.44	0.46	0.01	0.02
Forage OM	0.99	0.97	0.04	0.64
Concentrate OM	0.40	0.41	0.01	0.02
Total DM	1.58	1.58	0.04	0.99
Total OM	1.39	1.38	0.04	0.93
NDF	0.88	0.89	0.03	0.69
ADF	0.48	0.50	0.02	0.33
Starch	0.12	0.12	0.01	0.62
DDM[1]	0.81	0.76	0.02	0.17
GE, Mcal/d	35.89	35.17	1.23	0.67
DE, Mcal/d	17.54	16.11	0.65	0.13
Rate of Intake, g/min	160.00	130.00	0.01	0.05

[1]DDM = digestible dry matter

Rate of Intake

There was a significant effect of treatment ($P = 0.05$) with the rate of intake slower for the extruded nugget than the pelleted concentrate.

Concentrate was fed on a percent body weight basis for each horse. Horses fed the pellet received what looked like a smaller amount of concentrate when compared to the extruded nugget due to the lower density of the nugget. These findings agreed with a study done by Shabi et al., (1999) who saw a decrease in corn density by 37.5%. Accordingly, horses receiving the extruded nugget had a greater volume to eat than their counter parts receiving the pellet and therefore were slower to consume their concentrate though each horse was fed at 0.05% BW.

Dry Matter Intake (DMI)

No significant effect of feed processing was seen on total DMI, hay DMI, or digestible DMI. However, grain DMI was significantly different ($P = 0.02$), and was higher for the extruded nugget than the pellet.

There was no difference on total DMI, hay DMI, and digestible DMI between the pellet and the extruded nugget. This contradicts findings of both Shabi et al. (1999) who observed a 7% decrease in DMI of cattle fed extruded feed and Gaebe et al. (1998) who observed a decrease in both DMI and digestible DMI in steers fed the extruded feed. The Gaebe et al. (1998) study in steers also saw a decrease in DE intake, in our study this was not observed between the treatments. Looking at the concentrate DMI in this study the increase observed in the extruded nugget results from the extruded nugget having a

greater DM than the pellet and horses were fed on an as-fed basis. This also explains why there was greater OM intake in concentrate for the extruded nugget than the pellet.

Starch Intake

There was no difference seen observed dietary treatments for starch intake values ($P = 0.62$). Lack of differences in starch intake was expected due to the values of starch between the concentrates being similar and the same hay being fed to all the animals thus minimizing the starch intake variability between treatments.

Digestion Parameters

Table 3. Least square treatment means of digestion parameters including DM, OM, NDF, ADF, Starch, and GE digestion in horses receiving a pelleted concentrate (PEL) and a extruded nugget (EXT).

	Treatment			
Item	PEL	EXT	SEM	*P*-value
Total Tract Digestion, %				
DM	51	48	0.01	0.03
OM	51	48	0.01	0.03
NDF	41	40	0.01	0.34
ADF	33	31	0.01	0.25
Starch	90	87	0.01	0.02
GE	49	46	0.01	0.03

Dry Matter Digestion (DMD) and Organic Matter Digestion (OMD)

There was a significant effect observed for DMD between the dietary treatments ($P = 0.03$), with DMD being greater for the pellet concentrate than the extruded nugget. A significant effect ($P = 0.03$) was observed for OMD driven by dietary treatment with OMD being greater for the pellet than the extruded nugget.

Apparent DMD in the current study differs from the findings of Cheng and Hardy (2003) where digestion in rainbow trout increased by an average of 15.7 % between the four different diets. Similar findings were reported by Gaebe et al. (1998) in steers fed extrusion processed feed where digestion was 4.4% higher for extruded than dry rolled feed. Although extrusion processing typically improves nutrient utilization of feedstuffs, if the processing technique is too extreme nutrient quality can be affected adversely (Masatcioglu et al., 2014). The current study also showed contradicting findings of OMD. Sun et al. (2006) observed an increase in OMD from 86% to 94% between a raw and extruded diet of potato starch and wheat bran.

Starch Digestion

There was a significant effect of dietary treatment on starch digestion with digestion being greater for the pellet compared to the extruded nugget ($P = 0.02$).

Gaebe et al. (1998) observed an increase in total tract starch digestibility of 96.4% in extruded grains versus 85.5% in dry rolled. The decrease in apparent starch digestion in the current study may be attributed to the feed processing of the extruded nugget as some conditions of heat can allow amylose recrystallization and cause the starch molecule to be less susceptible to enzymatic digestion (NRC,2007). Additionally, differences in our study and Gaebe et al. (1998) most likely result from the differences in digestive physiology of foregut and hindgut fermenters. Rapid starch fermentation in the rumen of cattle may have allowed for increased digestion and utilization compared to enzymatic digestion in the small intestine of equines. Observations from the current study allow us to note that since starch digestion was affected by extrusion processing

with digestibility of 90% for pellet and 87% for extruded nugget and DE was not significantly affected by processing ,with pellet having 17.54 Mcal/d and extruded with 16.11 Mcal/d , these similar DE values between the dietary treatments may be attributed to the other nutrients in the ration not being negatively impacted by the extrusion feed process to provide energy for the animals. This coincides with the NRC (2007) where one of the factors that impacts the DE is the digestibility of the energy-containing components, which can be altered by the processing of the components.

Gross Energy (GE) Digestion

In accordance with previous measures of nutrient availability, GE digestion was greater for the pellet than the extruded nuggets. Lower GE in the extruded feed may explain the differences observed in digestion as more energy was available to the horse fed the pellet.

Glycemic Index Parameters

Glucose and Insulin Response

Dietary treatment did not affect glucose and insulin concentrations ($P = 0.81$, $P = 0.81$, respectively). A trend towards a time effect was observed for glucose ($P = 0.07$, Fig. 2) demonstrated by increases to 210, decreases to 420, and increasing to 480 for both PEL and EXT. There is a significant effect of time on insulin ($P < 0.01$, Fig. 3) observed by values increasing over time. There was no significant effect of treatment on area under the curve (AUC) for glucose and insulin (P = 0.67, P = 0.51, Table 4) or for peak glucose and insulin concentrations (P = 0.47, P = 0.59, Table 4).

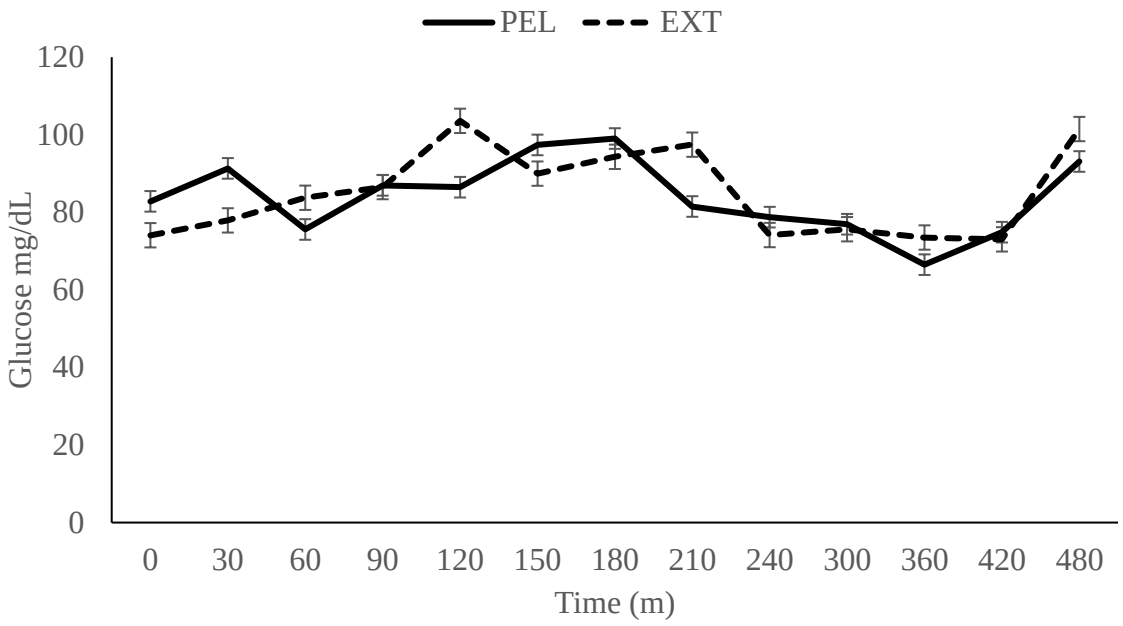

Figure 1. Mean plasma glucose in response to feeding dietary treatments, PEL (pellet) and EXT (extruded).

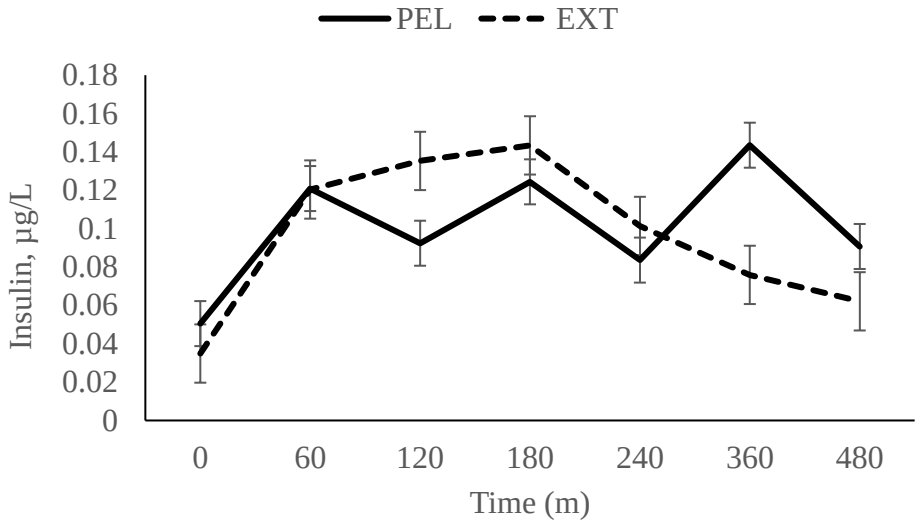

Figure 2. Mean plasma insulin in response to feeding dietary treatments, PEL (pellet) and EXT (extruded).

Table 4. Least square treatment means of glucose and insulin parameters including area under the curve (AUC) and peak levels in horses receiving a pelleted concentrate (PEL) and an extruded nugget (EXT).

	Treatment			
Item	PEL	EXT	SEM	*P*-value
AUC Glucose	181.40	187.10	9.95	0.67
AUC Insulin	60.46	53.77	7.36	0.51
Peak Glucose (mg/dL)	139.20	127.90	13.93	0.47
Peak Insulin (μg/L)	0.23	0.19	0.04	0.59

The lack of significance seen in glucose and insulin between dietary treatments can help explain the higher starch digestion between the pellet and concentrate by showing that starch digestion must have been compromised by factors such as processing method instead of just digestion site. These results can also be confounded by intake time, gastric emptying, digestion, and rate of absorption (Hoffman 2009). As seen in a study by Stull and Rodiek (1987) the current study showed plasma glucose concentrations were obtained between 2-3-h after ingestion of the meal with the peak for EXT happening before PEL. However, when looking at peak insulin levels which have been reported to normally appear 4-h after ingestion of a meal (Stull and Rodiek 1987) the levels in this study appeared for EXT at 2-h after the meal. AUC in the current study showed for EXT compared to PEL the AUC was greater for glucose but was lower for insulin. These findings are contradicting to findings from Gordon et al. (2008) who observed that the AUC was greater for extrusion than pelleting for both glucose and insulin values. Looking at this study compared to the current study the same thing as we saw in AUC was true for peak glucose and insulin value with the current study being higher for extrusion for only peak glucose and not peak insulin (Table 4).

Glycemic index between PEL and EXT was determined by using PEL as the standard an setting the glycemic index value to 100. The glycemic index was then determined for EXT by taking the percent difference of AUC EXT from AUC of PEL. The glycemic index for EXT was 103.11 and PEL was 100. This shows that the processing method did alter how the starch was digested pre-cecally due to the glycemic index being higher for the EXT than PEL. However, glycemic index may be effected by multiple things such as meal size, enzymatically digestible starch concentrations, feed processing, and rate of intake (Rodiek and Stull 2007). It is interesting to note that the glycemic index was greater for the EXT than the PEL which may have been effected by the rate of intake being slower for EXT than PEL allowing for more pre-cecal digestion for the EXT than the PEL due to continuous food entering the digestive tract for the duration of eating instead of only a couple blouses of pellet entering at once.

CHAPTER V

SUMMARY

Previous studies in livestock have suggested the potential benefit of extrusion feed processing on nutrient utilization. The effect of extrusion feed processing has been looked at in horses from a glycemic index standpoint, however, little information is known on the apparent digestion effects of extrusion feed processing in horses.

The current study indicated that the extrusion feed processing decreased the apparent digestibility of DMD, OMD, starch digestion, and GE digestion. In addition, there was a decrease in the concentrate rate of intake for the extruded feed than the pelleted feed. Dietary treatment did not effect DMI, hay DMI, DDMI, starch intake, GE intake, NDF intake, ADF intake, NDF digestion, ADF digestion, DE values, glucose response, insulin response, AUC glucose and insulin, and peak glucose and insulin values.

Based on these results, further studies are needed to evaluate single nutritional components response to extrusion feed processing on apparent digestion since in this study there was not a difference in fiber digestion meaning the change is in a different nutritional component. Also, there would be a benefit to considering the different extrusion methods (temperature, die size, single or twin screw) and determining which method maximizes the benefits without over processing the feedstuff and decreasing the availability of nutrient utilization in the body. This could be done by making up multiple protocols and performing a digestibility study to compare the results from the multiple

methods. It would also be interesting to do a glycemic index for different processing methods of feed with the same ingredients but using the same individual horses for each processing method to reduce animal variability in glucose and insulin response to the feeds.

REFERENCES

Alonso, R., L. A. Rubio, M. Muzquiz, and F. Marzo. 2001. The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. Anim. Feed Sci. Tech. 94:113.

Brouns, F., I. Bjorck, K. N. Frayn, A. L. Gibbs, V. Lang, G. Slama, and T. M. S. Wolever. 2005. Glycaemic index methodology. Nutr Res Rev. 18: 145-171. doi: 10.1079/NRR2005100.

Cheng, Z. J. and R. W. Hardy. 2003. Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). Aquacult. Nutr. 9:77-83

Cheftel, J. C. 1986. Nutritional effects of extrusion-cooking. Food Chem. 20:263-283.

Cochran, R. C. and M. L. Galyean. 1994. Meaurements of in vivo forage digestion by ruminants. In: G. Fahey, editor, Forage quality, evaluation, and utilization. ASA, Madison, WI. p. 613-643.

EL-Khalek, E. and G. P. J. Janessens. 2010. Effect of extrusion processing on starch gelatinization and performance in poultry. World Poultry Sci. J. 66:53-63. doi: 10.1017/S0043933910000073.

Gaebe, R. J., D. W. Sanson, I. G. Rush, M. L. Riley, D. L. Hixon, and S. I. Paisley. 1998. Effects of extruded corn or grain sorghum on intake, digestibility, weight gain, and carcasses of finishing steers. J. Anim. Sci. 76:2001-2007.

González-Alvarado, J. M., E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci. 86:1705-1715.

Goodman, B. E. 2010 Insights into digestion ad aborption of major nutrients in humans. Adv. Physiol. Ed. 34:44-53. doi: 10.1152/advan.00094.2009.

Gordon, M. E., M. L. Jerina, R. H. Raub, K. E. Davison, and J. K. Young. 2008. The effects of feed form on consumption time and glucose and insulin response to a concentrate meal in equine. J. Vet. Sci. 28: 289-294.

Harbour, L. E., L. M. Lawrence, S.H. Hayes, C. J. Stine, and D. M. .Powell. Concentrate composition, form, and glycemic response in horses. Proceedings 18th Equine

Nutrition and Physiology Symposium. 2003: 329-330.

Harris, P. and R. J. Geor. 2009. Primer on dietary carbohydrates and utility of the glycemic index in equine nutrition. Vet Clin Equine. 25: 23-37. doi: 10.1016/j.cveq.2009.01.006.

Hilton, J. W., C. Y. Cho, and S. J. Slinger. 1981. Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*salmo gairdneri R.)* Aquaculture 25:185-194.

Hoekstra, K.E., K. Newman, M.A.P. Kennedy, and J.D. Pagan. 1999. Effect of corn processing on glycemic responses in horses. Proceedings of the XVI Equine Nutrition and Physiology Symposium of the Equine Nutrition and Physiology Society

Hoffman, R. M. 2009. Carbohydrates metabolism and metabolic disorders in horses. R. Bras. Zootec. 32:270-276.

Julliand, V., A. De Fombelle, and M. Varloud. 2005. Starch digestion in horses: the impact of feed processing. Livest. Sci. 100:44-52. doi:10.1016/j.liveprodsci.2005.11.001

Kronfeld, D. S., K. H. Treiber, T. M. Hess, and R. C. Boston. 2005. Insulin resistance in the horse: definition, detection, and dietetics12. J. Anim. Sci. 83(Suppl13):E22-E31. doi:10.2527/2005.8313_supplE22x

Mahasukhonthachat, K., P. A. Sopade, and M. J. Gidley. 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. J Cereal Sci. 51:392-401. doi: 10.1016/j.jcs.2010.02.008.

Masatcioglu, T. M. , P. K. W. Ng, and H. Koksel. 2014. Effects of extrusion cooking conditions and chemical leavening agents on lysine loss as determined by furosine content in corn based extrudates. J. Cereal Sci. 60:276-281. doi:10.1016/j.jcs.2014.06.008.

McLean, B. M. L., J. J. Hyslop, A. C. Longland, D. Cuddeford, and T. Hollands. 2000. Physical processing of barley and its effects on intra-caecal fermentation parameters in ponies. Anim. Feed Sci. Tech. 85: 79-87.

Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Anim. Feed Sci. Tech. 108: 95-117. doi; 10.1016/S0377-8401 (03) 00163-9.

NRC. 2007. Nutrient requirements of horses. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Ralston, S. L. 2007. Evidence-based equine nutrition. Vet Clin Equine. 23: 365-384. doi: 10.1016/j.cveq.2007.03.007.

Rodiek, A. V. and C. L. Stull. 2007. Glycemic index of ten common horse feeds. J. Vet. Sci. 27: 205-211. doi: 10.1016/j.jevs.2007.04.002.

Rosenfeld, I. and D. Austbo. 2009. Effect of type of grain and feed processing on gastrointestinal retention time in horses. J. Anim. Sci. 87:3991-3996. doi:10.2527/jas.2008-1150.

Rosenfeld, I. and D. Austbo. 2009. Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. Anim. Feed Sci. Tech. 150: 249-258. doi: 10.1016/j.anifeedsci.2008.09.002.

Rowe, J. B., M. Choct, and D. W. Pethick. 1999. Processing cereal grains for animal feeding. Aust. J. Agric. Res. 50:712-736. doi: 10.1071/AR98163.

Serrano, X. 1997. The extrusion-cooking process in animal feeding. Nutritional implications.CIHEAM. 26:107-114.

Shabi, Z. , I. Bruckental, S. Zamwell, H. Tagari, and A. Arieli. 1999. Effects of extrusion of grain and feeding frequency on rumen fermentation, nutrient digestibility, and milk yield and composition in dairy cows. J. Dairy Sci. 82:1252-1260

Singh, S., S. Gamlath, and L. Wakeling. 2007. Nutritional aspects of food extrusion: a review.Int.J. Food Sci. Tech. 42:916-929. doi: 10.1111/j.1365 2621.2006.013909.x

Sun, T., H. N. Laerke, H. JØrgensen, K. E. B. Knudsen. 2006. The effect of extrusion cooking of different starch sources on the in vitro and in vivo digestibility in growing pigs. Anim. Feed Sci. Tech. 131:66-85. doi: 10.1016/j.anifeedsci.2006.02.009

Svinus, B., A. K. Uhlen, and O. M. Harstad. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch; a review. Anim. Feed Sci. Tech. 122:303-320.doi:10.1016/j.anifeedsci.2005.02.025.

Tran, Q. D., W. H. Hendricks, and A. F. B. van der Poel. 2008. Effects of extrusion processing on nutrients in dry pet food. J. Sci. Food Agric. 88:1487-1493. doi: 10.1002/jsfa.

Van Rooijen, C., G. Bosch, A. F. B. van der Poel, P. A. Wierenga, L. Alexander, and W. H. Hendriks. 2013. The millard reaction and pet food processing: effects on nutritive value and pet health. Nutr. Res. Rev. 26:130-148. doi: 10.1017/S0954422413000103.

Van Weyenberg, S., J. Buyse, and G. P. J. Janssens. 2007. Digestibility of a complete ration in horses fed once or three times a day and correlation with key blood parameters. Vet. J. 173: 311-316.

Vervuert, I. and M. Coenen. 2005. Factors affecting glycaemic index of feds for horses. Pferdeheilkunde. 21:79-82.

Zeyner, A., C. Hoffmeister, A. Einspainer, J. Gottschalk, O. Lengwenat, and M. Illies. 2006. Glycaemic and insulinaemic response of quarter horses to concentrates high in fat and low in soluble carbohydrates. Equine Vet J. Suppl. 36: 643-647.